SECTION ON MICROBIOLOGY*

*Abstracts of Papers***

Presented at the Meeting, January 19, 1955, at The New York Academy of Medicine

The Immunological Properties of the Isolated Vi and O Antigens of Salmonella Typhosa

MAURICE LANDY

Army Medical Service Graduate School, Washington, D.C.

Among the antigens of Salmonella typhosa thus far identified, the Vi and 0 antigens are considered the two most essential in determining its pathogenic and immunogenic character. The 0 antigen is held to be largely responsible for the toxic attributes of the organism while the Vi antigen appears to be required for the invasive activities of virulent typhoid bacilli. The Vi antigen in the absence of the 0 is sufficient to protect mice effectively against infection with virulent strains of S. typhosa. In recent years this laboratory has been concerned with the improvement of antityphoid immunizing agents and major emphasis has been directed toward the isolation and characterization of the Vi and 0 antigens with a view toward their ultimate use as a purified substitute for typhoid vaccine.

Employing an ethanol-salt fractionation procedure Vi antigen was isolated from several V form Enterobacteriaceae including certain strains of Escherichia coli, Paracolobactrum ballerup and S. typhosa. The isolated antigen is a viscous acidic polymerized polysaccharide with a high content of acetyl groupings and probably is composed of units of N-acetylaminohexuronic acid. The purified Vi antigen, derived from E. coli, exhibits a high degree of serological activity and specificity in hemagglutination, complement-fixation and quantitative precipitin tests with Vi antibody. It is markedly effective in the protection of mice against challenge with virulent strains of S. typhosa, and is antigenic for mice, rabbits and man, evoking the production of antibody reactive with Vi antigen and Vi strains of S. typhosa. In addition such antibody possesses mouse protective potency. The antigen is as effective as Vi typhoid bacilli in absorption of mouse protective potency from Vi antisera. Approximately 95 per cent of human beings receiving a subcutaneous injection of 40 micrograms develop Vi antibody while larger amounts or multiple injections of antigen do not appear to further increase antibody levels. Antibody developed in response to a single injection may persist for periods up to two years in contrast to the rather rapid disappearance of Vi antibody following recovery from typhoid fever.

The somatic or 0 antigen (endotoxin) of S. typhosa was isolated from the 0 variant strain 0901 by extraction of viable bacilli with trichloroacetic acid and fractionation of the extract by ethanol-salt and am-

* Thomas P. Magill, Chairman, Maclyn McCarty, Secretary.

** Manuscript received June 1955.

monium sulfate procedures. The purified antigen thus obtained is a phosphorylated lipopolysaccharide containing approximately 66 per cent polysaccharide, 33 per cent lipid and only 0.6 per cent nitrogen, most of the latter being attributable to hexosamines. This lipopolysaccharide shows high activity and specificity in various *in vitro* immunological procedures. It completely precipitates 0 agglutinins from typhoid antisera, and agglutinin nitrogen and precipitable antibody nitrogen values for such sera are in good agreement. The serological specificity and agglutinative activity of erythrocytes coated with the lipopolysaccharide are entirely comparable to those of intact typhoid bacilli. The antigen protects mice against challenge with the 0901 and Panama Carrier strains of S. typhosa and is strikingly effective in evoking antibody production in rabbits and in man. The isolated lipopolysaccharide also possesses to a high degree those biological properties, such as lethality for mice and rabbits, pyrogenicity, and activity as a preparing and provoking agent in the local and generalized Shwartzman reaction, commonly attributed to the endotoxins of gram-negative bacteria.

When compared with the conventional typhoid vaccine in field trials in human beings, the isolated Vi and 0 antigens administered as a single injection of 40 and 20 micrograms each respectively, stimulate significantly greater 0 antibody levels and far greater Vi responses than a series of three injections of typhoid vaccine, indicating that on the basis of specific antibody responses, the activity of the purified antigens is superior to that of the bacillary vaccine.

Infection and Immunity in Experimental Typhoid Fever

GEOFFREY EDSALL

Army Medical Service Graduate School, Washington, D.C.

Although immunization against typhoid fever has been carried out on a large scale for a half century, no controlled studies on its effectiveness in man have been recorded to date. Extensive studies carried out in small laboratory animals have provided a mass of information on the relative capacities of different types of typhoid vaccine to protect these animals (e.g., mice or guinea pigs) from systemic infection induced by parenteral inoculation of typhoid bacilli. Much information has been accumulated on antigens of the typhoid bacillus which can be demonstrated either by test-tube techniques or by challenge and protection experiments in small animals.

However, the analogy between the mouse and man is not sufficiently close to justify transferring conclusions from one to the other. Since a number of different types of typhoid vaccine have had their strong advocates and opponents, and since several recent experiences (particularly those concerning British troops in the Middle East) have cast doubt upon the effectiveness of typhoid vaccine, it has appeared necessary to reexamine infection and immunity in typhoid fever, using primates as the experimental animal. Since it is not practical to carry out such experiments in man, the chimpanzee has been employed in accordance with successful findings reported 40 years ago by Metchnikoff and Besredka. Our group has established oral infection in chimpanzees, resulting in the development of a period of fever, bacteremia, and pathological changes essentially identical to those observed in human typhoid fever. Immunization with whole vaccine, followed by a challenge with a homologous strain of S. typhosa shortly after a booster dose of vaccine, has resulted in apparent complete protection of the immunized animals, in contrast to an approximately 80 per cent infection rate observed repeatedly in the unimmunized animals. On the other hand, in one preliminary experiment purified Vi antigen yielded only partial protection, and purified typhoid O antigen gave no protection.

Antibody studies on control and immunized animals have shown the expected responses; instances of subclinical infection and the carrier state have been observed, and histopathological studies of infected animals are being carried forward. Problems as yet unsolved, such as:

a. How long does vaccine protect?
b. Will vaccine protect against heterologous strains?
c. Will a different type of vaccine protect?
d. And can protection be achieved by isolated antigens?

will be pursued in the near future.

These experiments in protection of chimpanzees with typhoid vaccine, along with a controlled field study of typhoid vaccine carried out in Europe this year, may eventually help to clear up the recent widely expressed doubts concerning the effectiveness of typhoid vaccine.